Purification and Subunit Analysis of Wheat-Germ Ribonucleic Acid Polymerase II

By JEROME J. JENDRISAK and WAYNE M. BECKER
*Department of Botany and Institute of Plant Development, University of Wisconsin, Madison,
Wis. 53706, U.S.A.*

A procedure is described for the purification of the α-amanitin-sensitive DNA-dependent RNA polymerase [EC 2.7.7.6] from wheat germ. Solubilization of the enzyme activity was achieved by sonication of a crude extract in a high-salt buffer. Purification involved precipitation with protamine sulphate and $(NH_4)_2SO_4$, chromatography on DEAE-cellulose and phosphocellulose, and sucrose gradient centrifugation. Under denaturing conditions the enzyme dissociated into five polypeptides with molecular weights and molar ratios of 220000 (0.9), 170000 (0.1), 140000 (1.0), 45000 (0.2), and 40000 (0.4). Approx. 1 mg of purified RNA polymerase was obtained as a routine from 100g of starting material.

DNA-dependent RNA polymerases have been purified from a number of eukaryotic organisms in recent years, since problems originally encountered in solubilizing the enzymes have largely been overcome (Roeder & Rutter, 1969; Blatti *et al.*, 1970). Eukaryotes have multiple nuclear RNA polymerases, which can be separated on DEAE-cellulose (Sephadex) columns (Blatti *et al.*, 1970; Roeder & Rutter, 1970). Polymerase I is nucleolar in origin (Roeder & Rutter, 1970) and probably synthesizes ribosomal RNA exclusively (Blatti *et al.*, 1970). Polymerase II is nucleoplasmic (Roeder & Rutter, 1970) and synthesizes the bulk of the remaining RNA (Blatti *et al.*, 1970). Other nuclear RNA polymerases (III and IV) have been isolated from some organisms (Blatti *et al.*, 1970), but they are not as well-characterized as polymerases I and II and their roles remain obscure. Polymerase II can be distinguished from all other forms of RNA polymerases by its sensitivity to the mushroom toxin α-amanitin, which binds to the enzyme (Jacob *et al.*, 1970) and inhibits its activity (Stirpe & Fiume, 1967).

Because polymerase I is present in smaller quantities and its activity is generally far less stable than polymerase II from most tissues (Chesterton & Butterworth, 1971), there are only a few reports of its complete purification and subunit composition (Kedinger *et al.*, 1971; Chambon *et al.*, 1970). Polymerase II, on the other hand, has been purified to apparent homogeneity from a number of mammalian sources, including calf thymus (Weaver *et al.*, 1971; Kedinger & Chambon, 1972) and rat liver (Weaver *et al.*, 1971) nuclei, and KB (Sugden & Keller, 1973) and HeLa (Sugden & Sambrook, 1970) cells, and its subunit structure determined.

According to Weaver *et al.* (1971), calf thymus and rat liver both contain two forms of polymerase II, each with four distinct subunits. One enzyme (IIA) has subunits with molecular weights and molar ratios of 190000 (1), 150000 (1), 35000 (1) and 25000 (1); the other enzyme (IIB) has a composition of 170000 (1), 150000 (1), 35000 (1) and 25000 (1). It has been postulated (Weaver *et al.*, 1971) that the 170000-mol. wt. subunit of enzyme IIB is derived from the 190000-mol. wt. subunit of enzyme IIA by proteolytic cleavage. It is, however, not yet clear whether both forms of the enzyme exist *in vivo* or whether enzyme IIB is an artifact of the purification procedure. The initial findings of Weaver *et al.* (1971) on the subunit composition of RNA polymerase II have since been confirmed and extended by other workers (Kedinger & Chambon, 1972), so that there now appears to be good general agreement on the molecular weights and molar ratios of the large subunits of mammalian polymerases. However, discrepancies still exist as to the number, molecular weights, molar ratios, and significance of the smaller polypeptides (Jacob, 1973).

Multiple nuclear RNA polymerases have also been solubilized and partially purified from several plant sources (Strain *et al.*, 1971; Mondal *et al.*, 1972; Horgen & Key, 1973; Jendrisak & Becker, 1973), but reports of complete purification and subunit composition are few (Ganguly *et al.*, 1973; Mullinix *et al.*, 1973). We have previously reported some of the enzymic properties of the two partially purified RNA polymerases from wheat germ (Jendrisak & Becker, 1973). Polymerase I, which makes up less than 10% of the total RNA polymerase activity in wheat germ, is eluted from DEAE-cellulose at low salt concentrations $[0.1\,\text{M-}(NH_4)_2SO_4]$, prefers denatured to native DNA by a factor of 2, is more active with Mn^{2+} than with Mg^{2+}, has maximal activity at low salt concentrations [less than 0.05M-$(NH_4)_2SO_4$] and is α-amanitin-insensitive. Polymerase II, which accounts for more than 90% of

the total RNA polymerase activity in wheat germ, is eluted at higher salt concentrations from DEAE-cellulose [0.2M-$(NH_4)_2SO_4$], prefers denatured DNA by a factor of 9, is more active with Mn^{2+} than Mg^{2+}, has a higher salt requirement [optimum at 0.125M-$(NH_4)_2SO_4$] and is α-amanitin-sensitive. Both enzymes have pH optima at 8.0–8.2 in a 0.05M-Tris buffer at 25°C. We have not observed other RNA polymerase species from wheat germ in any of our results.

We report here further purification of polymerase II from wheat germ and compare its subunit structure with the corresponding enzymes isolated from other eukaryotic organisms. A preliminary report of this work has appeared elsewhere (Becker *et al.*, 1973).

Materials and Methods

Materials

Wheat germ. Freshly milled wheat germ (isolated embryos) was obtained from VioBin Corp., Monticello, Ill., U.S.A. This material can be stored in a cold-room (4°C) for more than 1 year with no loss in RNA polymerase activity.

Nucleotides. Unlabelled ribonucleoside triphosphates (ATP, CTP, GTP, and UTP) were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A. [5-^{3}H]UTP (26.9Ci/mmol) was a product of New England Nuclear Corp., Boston, Mass., U.S.A.

DNA template. Calf thymus DNA was purchased from Worthington Biochemical Corp., Freehold, N.J., U.S.A., and was heat-denatured just before use.

Buffers. Glass-double-distilled water was used in all buffers. All Tris-buffer pH values are reported at 25°C. All chemicals used in buffers were reagent grade.

(1) Grinding buffer contained 15mM-2-mercaptoethanol in Tris–HCl, pH8.1.

(2) Chromatography buffer contained 15mM-2-mercaptoethanol, 25% (v/v) glycerol and 0.1% Triton X-100 (Sigma) in 0.05M-Tris–HCl, pH8.1. The Triton X-100 was included to maintain solubility of lipoproteins which otherwise tend to precipitate during chromatography.

Methods

RNA polymerase assay. The standard assay mixture contained, in a final volume of 0.25ml: 2.5μmol of Tris–HCl, pH8.1; 0.25μmol of $MnCl_2$; 5μmol of $MgCl_2$; 12.5μmol of $(NH_4)_2SO_4$; 0.1μmol each of GTP, CTP and ATP; 1μCi of [5-^{3}H]UTP diluted with unlabelled UTP to a specific radioactivity of 1μCi/100pmol; 50μg of heat-denatured calf thymus DNA and 0.050ml of enzyme. The concentration of UTP was limiting in this assay mixture to

enhance incorporation of radioactive UTP. The reactions were initiated by adding nucleoside triphosphates last, incubated for 15min at 25°C, and terminated by adding 0.1ml of 0.1M-$Na_4P_2O_7$ (previously adjusted to pH7 with HCl). The RNA products were precipitated with 4ml of ice-cold 5% (w/v) trichloroacetic acid. After 5min at 0°C, the precipitates were collected on Whatman GF/4 filters (2.4cm) and washed under suction with five 4ml rinses of ice-cold 5% trichloroacetic acid. The filters were allowed to dry at room temperature (20°C), then incubated overnight at room temperature in scintillation vials containing 1ml of Soluene tissue solubilizer (Packard Instrument Co. Inc., Downers Grove, Ill., U.S.A.). To each vial 10ml of toluene-based scintillation solution (Omnifluor; New England Nuclear, Boston, Mass., U.S.A.) was added and radioactivity was assayed at 50% efficiency in a Packard 3385 liquid-scintillation system.

One unit of enzyme activity is defined as the amount catalysing the incorporation of 1pmol of labelled ribonucleoside triphosphate into acid-precipitable form in 15min under the above conditions. Specific activity is defined as units/mg of protein.

Column chromatography. DEAE-cellulose (Sigma; 0.85mequiv./g dry wt.; medium mesh) was prepared for use by the method of Burgess (1969). The column was packed at room temperature and equilibrated with 10 column volumes of 0.175M-$(NH_4)_2SO_4$ in chromatography buffer at 0–4°C.

Phosphocellulose (Sigma; 0.80 mequiv./g dry wt.; medium mesh) was also prepared by the method of Burgess (1969). The column was packed at room temperature and equilibrated with 25 column volumes of 0.05M-$(NH_4)_2SO_4$ in chromatography buffer at 0–4°C. The conductivity and pH were monitored to ensure complete equilibration.

Sucrose gradient centrifugation. Linear 5–15% (w/v) sucrose gradients were formed in chromatography buffer containing 0.1M-$(NH_4)_2SO_4$.

Gel electrophoresis. Sodium dodecyl sulphate-polyacrylamide gels were run as described by Laemmli (1970) with a 4% acrylamide stacking gel and an 8.75% acrylamide running gel. Gels were stained overnight in 0.02% Coomassie Brilliant Blue (Sigma) in methanol–acetic acid–water (5:1:5, by vol.) and diffusion-destained in 7.5% (v/v) acetic acid at room temperature.

Conductivity measurements. Salt concentrations were determined from conductivity measurements made with a Radiometer (Copenhagen, Denmark) conductivity meter (CDM2e).

Protein determinations. Protein was measured by the method of Lowry *et al.* (1951) after the precipitation step of Sugden & Keller (1973) to remove interfering substances. Small amounts of protein were determined by densitometry on sodium dodecyl sulphate-polyacrylamide gels stained with Coomassie

Blue, by using known amounts of *Escherichia coli* RNA polymerase as standards. Gels were scanned at 550nm with a Gilford 2400 recording spectrophotometer equipped with a gel-scanning attachment and peak areas determined with a planimeter. The band intensities (peak areas) were assumed to be proportional to the amount of protein in the bands since the amount of Coomassie Blue bound is approximately proportional to the number of peptide bonds present. These measurements may be subject to error however, since for some proteins this relationship is not valid (Fazekas de St. Groth *et al.*, 1963).

Purification of RNA polymerase II

Unless otherwise specified, all operations were carried out at 0–4°C.

Step 1: Homogenization. Wheat germ (100g) was blended for 2min at full speed in 500ml of grinding buffer with a Sorvall Omnimixer. After stirring for 10min, the homogenate was centrifuged at 5000*g* for 5min to yield approx. 400ml of supernatant (fraction 1).

Step 2: Sonication and protamine sulphate precipitation. For this 0.25vol. (100ml) of 2.5M-$(NH_4)_2SO_4$ in grinding buffer was added slowly with stirring to fraction 1, during which time the viscosity increased greatly. The mixture was sonicated in 75-ml batches in a 100-ml stainless-steel beaker with a Heat Systems Ultrasonics W-185E sonifier operated at full power with the standard tip for 2min. The temperature was maintained at 5–8°C with an ice bath. The viscosity of the extract was decreased such that drops formed at the tip of a pasteur pipette. The sonicated extract (500ml) was rapidly mixed with 4vol. (2000ml) of 0.075% protamine sulphate (Sigma, grade I) in grinding buffer and the resulting mixture centrifuged at 20000*g* for 20min. The pellet consisted mainly of nucleic acids and the clear supernatant solution contained the solubilized polymerase activity (fraction 2).

Step 3: $(NH_4)_2SO_4$ precipitation. RNA polymerase activity was precipitated from fraction 2 by adding solid $(NH_4)_2SO_4$ slowly with stirring, to 33% saturation. After an additional 15min of stirring, the precipitate was collected by centrifugation (20000*g* for 20min) and dissolved in chromatography buffer (approx. 250ml) to a final $(NH_4)_2SO_4$ concentration of 0.175M. The resulting solution was cleared by centrifugation (105000*g* for 2h, Spinco no. 30 rotor) to give fraction 3.

Step 4: DEAE-cellulose chromatography. Fraction 3 was applied to a DEAE-cellulose column (column volume, 100ml/100g of starting material) prepared as described previously. The sample was followed by 5 column volumes of 0.175M-$(NH_4)_2SO_4$ in chromatography buffer. Polymerase I fails to bind to the column

under these conditions and is present in the flow-through along with a slight amount of polymerase II activity, as determined by assaying in the presence and absence of α-amanitin (Henley & Co., N.Y., U.S.A.) at a concentration of 4μg/ml. A sharp peak of polymerase II activity (completely sensitive to α-amanitin) was eluted by increasing the $(NH_4)_2SO_4$ concentration in the chromatography buffer to 0.35M. The fractions containing most of the activity were pooled to give fraction 4.

Step 5: Phosphocellulose chromatography. Fraction 4 (approx. 40ml) was desalted on a 250-ml column of Sephadex G-25 (coarse grade) equilibrated with 0.05M-$(NH_4)_2SO_4$ in chromatography buffer. The polymerase-containing fractions were pooled and applied to a 20-ml phosphocellulose column equilibrated with 0.05M-$(NH_4)_2SO_4$ in chromatography buffer. The sample was followed by 5 column volumes of the same buffer and the polymerase activity was then eluted with 0.1M-$(NH_4)_2SO_4$ in chromatography buffer. The fractions containing most of the activity were pooled to give fraction 5.

Step 6: Sucrose gradient centrifugation. Portions (0.5ml) of fraction 5 were layered on 4.5ml sucrose gradients prepared as described above, and centrifuged at 220000*g* for 24h at 0–4°C (Spinco SW-50L rotor at 45000rev./min).

Results and Discussion

Purification procedure

A summary of the purification of RNA polymerase II from 100g of wheat germ appears in Table 1. Grinding the material in a low-salt buffer followed by a brief stirring extracts the chromatin, to which the polymerase activity is bound. Low-speed centrifugation (5000*g*) removes the gross debris (90% of the dry wt. of the wheat germ) without pelleting the chromatin. Increasing the salt concentration of the extract to 0.50M-$(NH_4)_2SO_4$ greatly increases the viscosity, owing to the high concentration of nucleohistone, and dissociates and solubilizes the chromatin constituents. Sonication greatly hastens the process and is necessary for maximal solubilization of the polymerase activity. Lowering the ionic strength of the sonicate to 0.1M-$(NH_4)_2SO_4$ (by dilution) allows the nucleohistone complex to re-form and precipitate. The polymerase does not reassociate with the chromatin at this salt concentration, but remains in the supernatant. Previously (Jendrisak & Becker, 1973) ultracentrifugation for several hours at high speeds was required to pellet completely the chromatin and ribosomes, but the inclusion of protamine sulphate in the diluting buffer allows a more efficient removal of nucleoproteins at lower centrifugal forces for shorter periods of time (20000*g* for 20min). The high loss in enzymic activity at this step may be due to

Table 1. *Summary of purification of RNA polymerase II*

This table summarizes the purification of RNA polymerase II from 100g of wheat germ. One unit of enzyme activity is defined as the amount catalyzing the incorporation of 1pmol of labelled ribonucleoside triphosphate into RNA in 15min under the assay conditions described in the text. Other experimental details are given in the text.

Fraction	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Yield (%)
1. Crude extract	10000	302†	0.03	100
2. Protamine sulphate supernatant	9000	111†	0.01	37
3. $(NH_4)_2SO_4$ precipitate	2500	125†	0.05	41
4. Pooled DEAE-cellulose peak fractions	6.2*	64	10	21
5. Pooled phosphocellulose peak fractions	1.0*	42	42	14
6. Pooled sucrose gradient peaks	0.8*	35	44	11

* The protein content of these fractions was determined by densitometric measurements of gels stained with Coomassie Blue, by using known quantities of *E.coli* RNA polymerase as standards.

† RNA polymerase II activity was determined in these fractions as the difference in the activities observed in the absence and presence of a saturating concentration of α-amanitin (4μg/ml).

removal of those polymerase molecules still bound to DNA fragments which are precipitable with protamine sulphate. Also, since protamine, like other polycations, is a potent inhibitor of RNA polymerase activity, the actual yield at this step may not be as low as the data indicate (Table 1), because an increase in total RNA polymerase activity is observed frequently after the subsequent $(NH_4)_2SO_4$ precipitation step which may remove any residual protamine.

$(NH_4)_2SO_4$ precipitation results in a five-fold purification of polymerase II and in a ten-fold concentration of the protein, which facilitates application to DEAE-cellulose. Recovery of activity is always greater than 90%.

The greatest single increase in specific activity in the entire purification procedure is obtained by DEAE-cellulose chromatography (Table 1, Fig. 1). As a routine, this step results in a 200-fold purification with at least 50% recovery of activity. A small amount of α-amanitin-sensitive polymerase activity is usually present in the flow-through of the DEAE-cellulose column. This small loss of polymerase II is tolerated since polymerase I and a great bulk of other contaminating proteins are separated from the major polymerase II peak in this way. A sodium dodecyl sulphate–polyacrylamide-gel profile of the pooled polymerase II peak from the DEAE-cellulose column is shown in Plate 1(*a*) (gel A).

Phosphocellulose chromatography (Fig. 2) results in another four-fold purification of polymerase II with greater than 60% recovery of activity (Table 1). As shown by the electrophoretic profile [Plate 1(*a*), gel B], the two high-molecular-weight polymerase subunits constitute the bulk of the protein in this fraction. The ability to recover high percentages of activity on phosphocellulose columns contrasts with the report of total loss in activity of polymerase II from maize (Strain *et al.*, 1971), but agrees with results obtained with various bacterial (Burgess, 1969) and mammalian (Weaver *et al.*, 1971) enzymes.
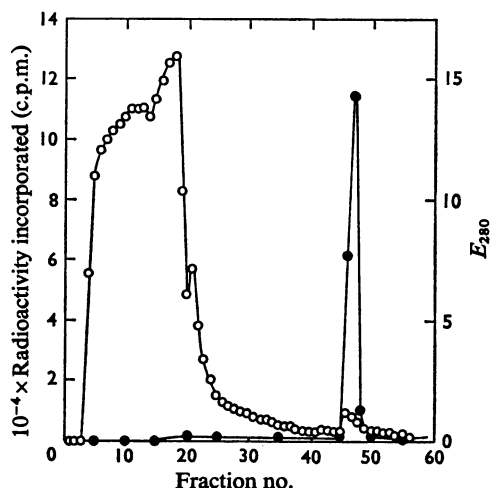

Fig. 1. *DEAE-cellulose column profile of wheat germ RNA polymerases*

Fraction 3 (2.5g of protein) was applied to a column (2.6cm×17cm) of DEAE-cellulose in chromatography buffer containing 0.175M-$(NH_4)_2SO_4$ and eluted as described in the text. Fractions (20ml) were collected at a flow rate of 100ml/h. Tubes 1–45 contained the flow-through material including polymerase I, which accounts for less than 10% of the applied polymerase activity and is eluted from DEAE-cellulose at salt concentrations considerably below 0.175M-$(NH_4)_2SO_4$ (Jendrisak & Becker, 1973). Tubes 46 and 47 contained most of the polymerase II activity eluted with 0.35M-$(NH_4)_2SO_4$ and were pooled to give fraction 4. ●, Radioactivity; ○, E_{280}

For maximum binding of polymerase to the phosphocellulose column, it is imperative that the flow rate should not exceed 2 column volumes/h. We have previously attempted to purify polymerase II on DNA–cellulose columns after DEAE-cellulose chromatography (Becker *et al.*, 1973). Many variations
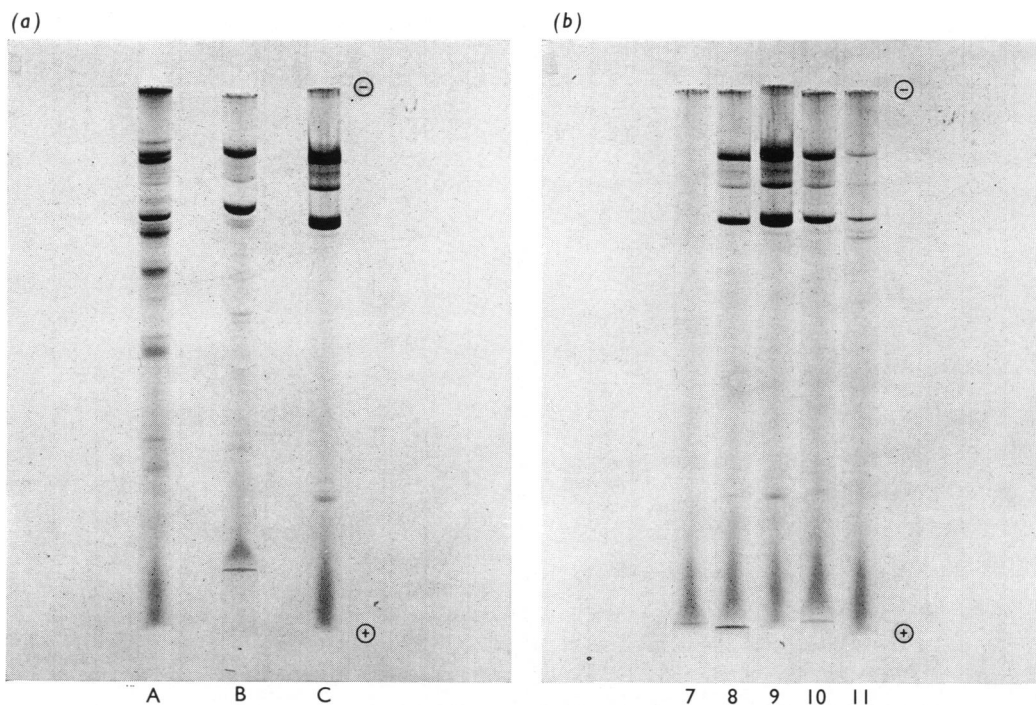
EXPLANATION OF PLATE I

Sodium dodecyl sulphate–polyacrylamide-gel analysis of (a) RNA polymerase II at various stages of purification and (b) sucrose-gradient fractions

(a) Gels were run as described in the text. (A) 12.6 μg of protein from the DEAE-cellulose column (fraction 4), (B) 8 μg of protein from the phosphocellulose column (fraction 5), (C) 17 μg of protein from tube 9 of the sucrose gradient (fraction 6). The origins are at the top and migration is toward the anode. (b) Gels (8.75% acrylamide) were run and stained as described in the text, with 0.1 ml samples of each fraction/gel. Numbers below the gels correspond to the sucrose-gradient fraction numbers in Fig. 3. The origins are at the top and migration is toward the anode.

EXPLANATION OF PLATE 2

Molecular-weight determination of wheat-germ RNA polymerase II subunits on sodium dodecyl sulphate–polyacrylamide gels

(A) 17 μg of wheat-germ polymerase, (B) 17 μg of wheat-germ polymerase and 11.5 μg of *E.coli* RNA polymerase, and (C) 11.5 μg of RNA polymerase. The subunits of *E.coli* RNA polymerase have the following molecular weights: β' 150000–165000; β 145000–155000; σ 85000–95000; α 39000–41000 (Burgess, 1971). The β' and β subunits are not resolved under our conditions of electrophoresis. Gels (8.75% acrylamide) were run and stained as described in the text. The origins are at the top and migration is toward the anode. A, A', B, C and D represent subunits in order of decreasing size.
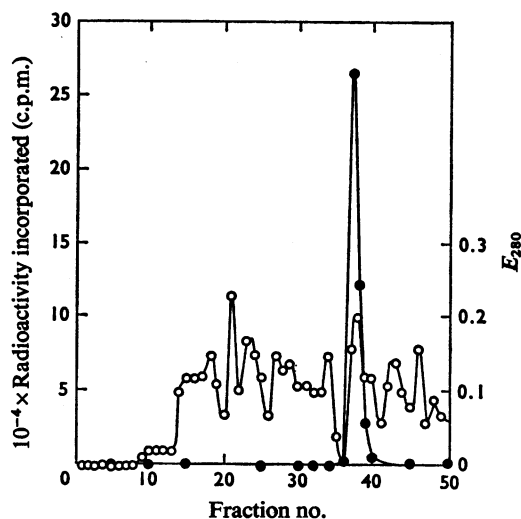
J. J. JENDRISAK AND W. M. BECKER

Fig. 2. *Phosphocellulose column profile of wheat germ RNA polymerase II*

Fraction 4 (polymerase II peak from the DEAE-cellulose column of Fig. 1; 6.2mg of protein) was desalted and applied to a column (2.0cm×6.7cm) of phosphocellulose in chromatography buffer containing $0.05\text{M}-(\text{NH}_4)_2\text{SO}_4$ and eluted as described in the text. Fractions (5ml) were collected at a flow rate of 20ml/h. Tubes 1–36 contained the flow-through material and tubes 37 and 38 contained most of the polymerase activity eluted with $0.1\text{M}-(\text{NH}_4)_2\text{SO}_4$, which were pooled to give fraction 5. ●, Radioactivity; ○, E_{280}.
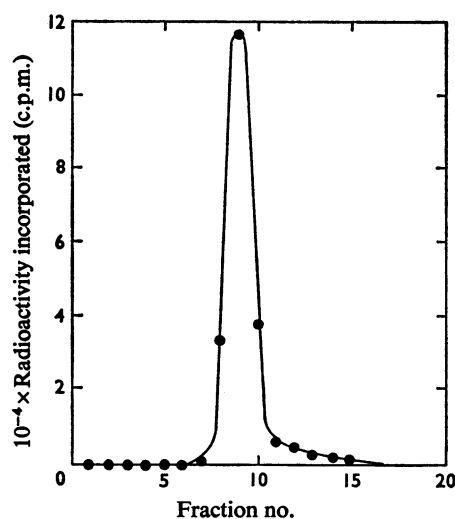

Fig. 3. *Sucrose gradient profile of wheat germ RNA polymerase II*

Samples (0.5ml) of fraction 5 (polymerase II peak from the phosphocellulose column of Fig. 2; 1mg of protein) were layered on 4.5ml sucrose gradients (5–15%) and centrifuged as described in the text. Fractions of volume 0.25ml were collected. Fraction 1 corresponds to the bottom of the tube. Owing to the low absorbance at 280nm, the amount of protein in these fractions was determined by densitometric analysis of the sodium dodecyl sulphate-polyacrylamide gels shown in Plate 1(b).

were made in flow rates and buffer composition, in attempts to obtain good recovery of enzyme activity with significant purification. In all cases, however, results with DNA-cellulose were inferior to those obtained with phosphocellulose chromatography under the above conditions.

Final purification of polymerase II is achieved by sucrose-density-gradient centrifugation (Fig. 3). The polymerase-containing fraction from the phospho-cellulose column can be applied directly to sucrose gradients without prior concentration or desalting. The additional purification achieved by this step is revealed in Plate 1(a) by comparing the electrophoretic profile of the applied sample (gel B) with that of the peak polymerase-containing fraction recovered from the gradient (gel C).

Shown in Plate 1(b) are the gel profiles for each fraction across the polymerase-containing region of the sucrose gradient. Comparison of enzyme activities (Fig. 3) with the gel profiles (Plate 1b) reveals the expected correlation between polymerase activity and protein content. Except for several contaminating polypeptides, which begin to appear in fraction 11 of the sucrose gradient, this correlation between

activity and protein content appears valid for each of the bands seen on the gels, suggesting that all of the polypeptides on the gels corresponding to fractions 8, 9 or 10 are components of the isolated enzyme, and that the polymerase has therefore been purified to homogeneity.

Yield, extent of purification, and stability

On the basis of the activity of the crude extract, the above procedure results in a recovery of approx. 10–12% of the original polymerase II activity, with an overall purification of about 1500-fold. The degree of purity shown in the gel patterns of Plate 1(b) suggests that most, if not all, of the protein recovered from the final purification step is RNA polymerase. Hence the yield by this procedure is about 0.8mg of purified RNA polymerase II from 100g of wheat germ.

In marked contrast with the stability of this enzyme after partial purification on DEAE-cellulose (Jendrisak & Becker, 1973), the fully purified enzyme prepared as described here is relatively unstable; half of the activity is lost within 1 week, even when

stored at −20°C. Preliminary evidence (electrophoresis under non-denaturing conditions) suggests that this loss of activity is accompanied by enzyme aggregation.

Subunit composition

Five bands are seen on electrophoresis of our purified enzyme under denaturing conditions (Plate 2, gel *a*). These are designated as subunits A, A′, B, C and D, in order of decreasing size. Assignment of approximate molecular weights to these subunits is based on the results shown in Plate 2, in which the subunit pattern of our enzyme (Plate 2, gel A) is compared electrophoretically with that of *E. coli* RNA polymerase prepared by the method of Burgess (1969), run with the wheat-germ enzyme (Plate 2, gel B) or in an adjacent tube (Plate 2, gel C). Molar

Fig. 4. *Densitometric analysis of wheat-germ RNA polymerase subunits*

Gel (A) of Plate 2 was scanned at 550nm by using the linear transport of the Gilford 2400 recording spectrophotometer. The origin of the gel is at the left and migration is toward the anode. Molar ratios of subunits were determined by measuring the areas under each peak with a planimeter and dividing those areas by the molecular weights of the corresponding polypeptides.

ratios for the subunits of the wheat-germ enzyme were determined by densitometric analysis of the gel profiles; a representative scan for our purified enzyme is shown in Fig. 4. The results of molecular-weight and molar-ratio estimations indicate that wheat-germ RNA polymerase II as isolated by the above procedure has an overall average subunit composition of: A 220000 (0.9); A′ 170000 (0.1); B 140000 (1.0); C 45000 (0.2) and D 40000 (0.4). For the higher-molecular-weight subunits, there appears to be reasonable agreement between our enzyme and the corresponding enzymes from both maize (Mullinix *et al.*, 1973) and mammalian sources (Weaver *et al.*, 1971; Kedinger & Chambon, 1972) although the lack of reliable polypeptide markers in this molecular-weight range precludes exact comparisons.

Since the molar ratio of $(A+A')/B$ for our enzyme is about 1, the interpretation cited above (Weaver *et al.*, 1971) for mammalian polymerase II may also be applicable to our enzyme; i.e., wheat-germ polymerase II may also exist in two forms, with subunit composition ABCD and A′BCD. Enzymes of these subunit compositions would have molecular weights of about 400000 and 450000. This agrees well with the molecular weights estimated for the native enzymes from other eukaryotic sources (Jacob, 1973). We have been unable to resolve our amanitin-sensitive RNA polymerase activity into two peaks chromatographically, but it may be significant that a shoulder of activity is almost always observed to be eluted for DEAE-cellulose slightly ahead of the major polymerase II activity when a linear salt gradient is used (Jendrisak & Becker, 1973). This may correspond to the postulated IIB form of the enzyme, which in any case would not be expected to account for more than about 10% of the total polymerase II activity.

Subunit A′ may arise from subunit A by proteolytic cleavage, as has been suggested for the corresponding polypeptides from other eukaryotic sources (Weaver *et al.*, 1971). If so, our data suggest a progressive, stepwise degradation, rather than a single specific scission, since numerous minor bands can be seen migrating between bands A and A′ on electrophoresis of large amounts of purified polymerase protein (Plates 1*a*, 1*b* and 2). That this heterogeneity may not be simply an artifact of the purification procedure is implied by the presence of these polypeptides in relatively constant proportions at various stages of enzyme purification (Plate 1*a*) and by our inability to demonstrate an enhanced appearance of polypeptide A′ or the intermediate polypeptides at the expense of polypeptide A on artificial aging of crude or purified enzyme preparations.

The small polypeptides C and D appear from our data to be subunits of the enzyme, since they co-purify with polypeptides A, A′ and B. This agrees well with the reported occurrence of two (Weaver *et al.*, 1971)
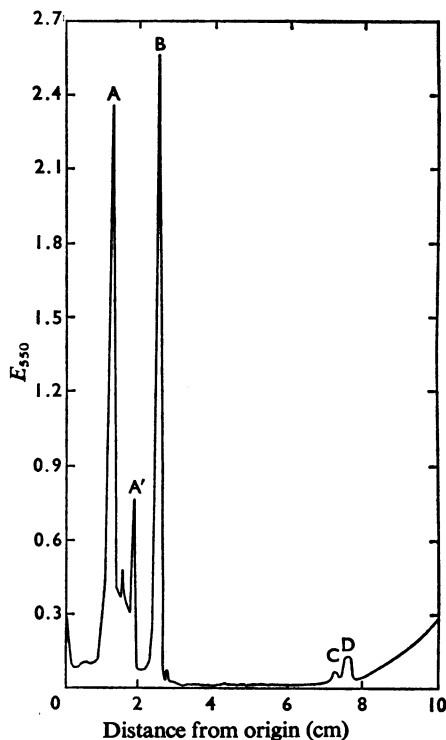
or three (Kedinger & Chambon, 1972) small subunits in RNA polymerase II from several mammalian sources, although the molecular weights that we estimate (45000 for C, 40000 for D) are somewhat higher than the values reported for the smaller subunits of the corresponding enzymes from rat liver and calf thymus (Weaver *et al.*, 1971) or from maize (Mullinix *et al.*, 1973). Moreover, our values are likely to be quite accurate, since they are based on a direct electrophoretic comparison of bands C and D with the 40000-dalton α subunit of *E. coli* RNA polymerase (Plate 2). Polypeptides with molecular weights less than that of the α subunit are in fact consistently absent from our purified enzyme preparations. Based on densitometry, subunits C and D of our purified polymerase have been assigned molar ratios of approx. 0.2 and 0.4, respectively. These values may, however, be subject to substantial error, owing to the difficulties of measuring such small amounts of protein adequately. Considerably more total polymerase protein would have to be applied to the gels for more accurate measurement of these values.

Conclusion

Wheat germ appears to be a good source for the isolation of RNA polymerase II. The material is inexpensive, available in large quantities, easy to store with little loss in activity, and is a rich source of enzyme (approx. 1mg of purified polymerase II/100g of wheat germ). The procedure described here is designed to minimize desalting, buffer exchange and protein-concentration steps, all of which are time-consuming and often lead to activity losses. The entire purification procedure takes about 3 days and yields an enzyme preparation with a limited number of subunits which agree reasonably well in size and molar ratios with those of other eukaryotic RNA polymerases. We reported previously that partially purified RNA polymerase II from wheat germ is strikingly similar to the corresponding enzymes from animal sources in its enzymic properties (Jendrisak & Becker, 1973). The data presented here extend the similarity to subunit composition as well. This agreement in physical and enzymic properties between plant and animal RNA polymerases and the ease with which polymerase II can be prepared in good yield from wheat germ, prompt us to suggest wheat germ as a potentially useful source of enzyme for further studies on the properties and regulation of eukaryotic RNA polymerases.

References

Becker, W. M., Jendrisak, J. J. & Glicklich, D. (1973) *Plant Physiol.* **51**, S38

Blatti, S. P., Ingles, C. J., Lindell, T. J., Morris, P. W., Weaver, R. F., Weinberg, F. & Rutter, W. J. (1970) *Cold Spring Harbour Symp. Quant. Biol.* **35**, 649–657

Burgess, R. R. (1969) *J. Biol. Chem.* **244**, 6160–6167

Burgess, R. R. (1971) *Annu. Rev. Biochem.* **40**, 711–740

Chambon, P., Gissinger, F., Mandel Jr., J. L., Kedinger, C., Gniazdowski, M. & Meihlac, M. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35**, 693–707

Chesterton, C. J. & Butterworth, P. H. W. (1971) *Eur. J. Biochem.* **19**, 232–241

Fazekas de St. Groth, S., Webster, R. G. & Datyner, A. (1963) *Biochim. Biophys. Acta* **71**, 377–391

Ganguly, A., Das, A., Mondal, H., Mandal, R. K. & Biswas, B. B. (1973) *FEBS Lett.* **34**, 27–30

Horgen, P. A. & Key, J. L. (1973) *Biochim. Biophys. Acta* **294**, 227–235

Jacob, S. T. (1973) *Progr. Nucl. Acid. Res. Mol. Biol.* **13**, 93–126

Jacob, S. T., Sajdel, E. M. & Munro, H. N. (1970) *Nature (London)* **225**, 60–62

Jendrisak, J. J. & Becker, W. M. (1973) *Biochim. Biophys. Acta* **319**, 48–54

Kedinger, C. & Chambon, P. (1972) *Eur. J. Biochem.* **28**, 283–290

Kedinger, C., Nuret, P. & Chambon, P. (1971) *FEBS Lett.* **15**, 169–174

Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Mondal, H., Mandal, R. K. & Biswas, B. B. (1972) *Eur. J. Biochem.* **25**, 463–470

Mullinix, K. P., Strain, G. C. & Bogorad, L. (1973) *Proc. Nat. Acad Sci. U.S.* **70**, 2386–2390

Roeder, R. G. & Rutter, W. J. (1969) *Nature (London)* **224**, 234–237

Roeder, R. G. & Rutter, W. J. (1970) *Proc. Nat. Acad. Sci. U.S.* **65**, 675–682

Stirpe, F. & Fiume, L. (1967) *Biochem. J.* **105**, 779–782

Strain, G. C., Mullinix, K. P. & Bogorad, L. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 2647–2651

Sugden, B. & Keller, W. (1973) *J. Biol. Chem.* **248**, 3777–3788

Sugden, B. & Sambrook, J. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35**, 663–669

Weaver, R. F., Blatti, S. P. & Rutter, W. J. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 2994–2999